PROCESS FOR THE PREPARATION OF 2-HYDROXYMETHYL-PYRROLIDINE 3, 4-DIOLS

deprotection of the corresponding N-protected 5-amino-5-deoxy-pentulose c) hydrogenation of the corresponding 5-amino-5-deoxy-pentulose.

ABSTRACT

A process for the preparation of 2-hydroxymethyl-pyrrolidine-3,4-diols of the formulae comprising the steps of a) biooxidation of N-protected aminotetraols of the formula b)
PROCESS FOR THE PREPARATION OF 2-HYDROXYMETHYL-PYRROLIDINE 3, 4-DIOLS

0001. The present invention relates to a process for the preparation of 2-hydroxymethyl-pyrrolidine-3,4-diols, to a process for the preparation of N-formyl-5-amino-5-deoxy-pentuloses as well as to the compounds N-formyl-1-amino-1-deoxy-D-arabinitol and N-formyl-1-amino-1-deoxy-L-arabinitol.


0003. Fleet et al. (Tetrahedron Lett. 1985, 26, 3127-3130) describe the preparation of (2R,3R,4R)—and (2S,3S,4S)-2-hydroxymethyl-pyrrolidine-3,4-diol from D-xylene. The disadvantages of both syntheses are that they involve a large number of steps and that the overall yields are low.

0004. Hung et al. (J. Org. Chem. 1991, 56, 3849-3855) describe the preparation of (2R,3R,4R)—2-hydroxymethyl-pyrrolidine-3,4-diol from 3-azido-propenyl-benzene. Ozonolysis of 3-azido-propenyl-benzene and subsequent enzymatically catalyzed aldol condensation of the obtained azido-acetaldehyde with dihydroxyacetone phosphate followed by dephosphorylation affords 5-azido-5-deoxy-D-xylulose, which is hydrogenated in the presence of palladium yielding (2R,3R,4R)-2-hydroxymethyl-pyrrolidine-3,4-diol. A disadvantage of the synthesis is that the hydrogenation is performed with a large amount of catalyst.

0005. EP 0 624 652 Al describes the preparation of 2-hydroxymethyl-pyrrolidine-3,4-diols and N-substituted derivatives thereof. 2-Hydroxymethyl-pyrrolidine-3,4-diol is prepared by microbial oxidation of N-benzyl-1-amino-1-deoxy-arabinitol using microorganisms of the genera Gluconobacter or Corynebacterium and subsequent hydrogenation using palladium as catalyst. N-Benzyl-1-amino-1-deoxy-arabinitol may be prepared from arabino and benzylamine. The disadvantages of the preparation of 2-hydroxyethyl-pyrrolidine-3,4-diol are that the microbial oxidation affords the oxidation product in low volume yield and that the catalytic hydrogenation is performed with large amounts of catalyst.

0006. It is an object of the present invention to provide an economic process for the preparation of 2-hydroxymethyl-pyrrolidine-3,4-diols. It is another object of the present invention to provide an economic process for the preparation of N-formyl-5-amino-5-deoxy-pentuloses and to provide new N-acylated 1-amino-1-deoxy-arabinitols.

0007. These objects are achieved by the processes according to claims 1, 12, and 17 and by the compounds according to claims 22 and 23.

0008. The process of the present invention for the preparation of 2-hydroxymethyl-pyrrolidine-3,4-diols comprises the steps of

0009. a) oxidizing an N-protected aminotetraol of the formula

0010. or a salt thereof, wherein R₁ is H, substituted or unsubstituted C₁₋₅-alkyl, substituted or unsubstituted C₂₋₅-alkenyl or OR₂; R₂ being unsubstituted C₁₋₅-alkyl, with a microorganism or a cell-free extract thereof to yield the corresponding N-protected 5-amino-5-deoxy-pentulose of the formula

0011. R₁ being defined as above,

0012. b) removing the N-protective group of said N-protected 5-amino-5-deoxy-pentulose (II) to yield the corresponding 5-amino-5-deoxy-pentulose of the formula

0013. and

0014. c) catalytically hydrogenating said 5-amino-5-deoxy-pentulose (III) to afford the corresponding (2R)—and/or (2S)-2-hydroxymethyl-pyrrolidine-3,4-diol of the formula

0015. The asterisks in the formulae I, II, III, IV and V denote chiral carbon atoms with defined configuration.

0016. N-Protected aminotetraols are selected from the group consisting of N-protected 1-amino-1-deoxy-D-arabi-

[0017] Preferred N-protected aminometaolts are N-protected 1-amino-1-deoxy-D-ribitol and N-protected 1-amino-1-deoxy-L-arabinobinitol.

[0018] The more preferred N-protected aminometaolts are N-protected 1-amino-1-deoxy-D-ribitol.

[0019] Salts of N-protected aminometaolts are e.g. the salts formed by treating N-protected amino-metabolts with strong mineral acids such as HCl.


[0022] The (2R)—and/or (2S)-2-hydroxymethyl-pyrrolidine-3,4-diol which corresponds to 5-amino-5-deoxy-D-ribose is (2R)-2-hydroxymethyl-pyrrolidine-3,4-diol. Accordingly, (2R,3R,4S)—and/or (2S, 3R,4S)-2-hydroxymethyl-pyrrolidine-3,4-diol correspond to 5-amino-5-deoxy-D-ribose, (2R,3S,4S)—and/or (2S,3S, 4S)-2-hydroxymethyl-pyrrolidine-3,4-diol correspond to 5-amino-5-deoxy-D-arabinobinitol, (2R,3R,4R)—and/or (2S, 3R,4R)-2-hydroxymethyl-pyrrolidine-3,4-diol correspond to 5-amino-5-deoxy-D-xylulose.

[0023] C₅₋₇-Alkyl may be branched or unbranched and may be substituted with at least one hydroxy group and/or halogen atom. Halogen atoms may be fluorne, chlorine, bromine or iodine. Examples of unsubstituted C₅₋₇-alkyl are methyl, ethyl, propyl, isopropyl, butyl, isobutyl and tert-butyl. The corresponding N-protective groups for R' being unsubstituted C₅₋₇-alkyl are acetyl, propionyl, butanoyl, isobutanoyl, pentanoyl, isopentanoyl and 2,2-dimethyl-propionyl (pivaloyl). The corresponding N-protective groups for R' being or R' being unsubstituted C₅₋₇-alkyl are methoxycarbonyl, ethoxycarbonyl, isopropoxycarbonyl, butoxycarbonyl, isobutoxycarbonyl and tert-butoxycarbonyl. Examples of substituted C₅₋₇-alkyl are chloromethyl, dichloromethyl, trichloromethyl, fluormethyl, difluoromethyl, trifluoromethyl, hydroxymethyl and 1-hydroxy-ethyl.

The corresponding N-protective groups for R' being substituted C₅₋₇-alkyl are chloroacetyl, dichloroacetyl, trichloroacetyl, fluoroacetyl, difluoroacetyl, trifluoroacetyl, hydroxyacetyl and 2-hydroxy-propanoyl.

[0024] C₅₋₇-Alkenyl may be branched or unbranched and may be substituted with at least one hydroxy group and/or halogen atom. Halogen atoms may be fluorne, chlorine, bromine or iodine. Examples of unsubstituted C₅₋₇-alkenyl are ethenyl, 1-propenyl, 2-propenyl, 1-methyl-2-propenyl, 2-methyl-1-propenyl, 1-butenyl, 2-butenyl and 3-butenyl. The corresponding N-protective groups for R' being unsubstituted C₅₋₇-alkenyl are acryloyl, crotonoyl, 3-butenoyl, 2-methyl-3-butenoyl, 3-methyl-2-butenoyl, 2-pentenoyl, 3-pentenoyl and 4-pentenoyl. Examples of substituted C₅₋₇-alkenyl are 1-chloroethenyl and 2-chloro-ethenyl. The corresponding N-protective groups for R' being substituted C₅₋₇-alkenyl are 2-chloroacryloyl and 3-chloroacryloyl.

[0025] For R' being H, the corresponding N-protective group is formyl.

[0026] Preferably, R' is H or substituted or unsubstituted C₅₋₇-alkyl. More preferably, R' is H.

[0027] The formyl protective group has the advantage that it provides a high solubility of the N-protected aminometaolts in aqueous medium. Thus the biooxidation can be performed at high concentrations of N-formyl aminometaolts affording a high volume yield of N-formyl-5-amino-5-deoxy-pentose. In the biooxidation of N-formyl-5-amino-5-deoxy-D-arabinobinitol volume yields of N-formyl-5-amino-5-deoxy-D-xylulose of up to 200 g/L can be achieved.

[0028] The N-protected aminometaolts can be prepared from the corresponding aminometaolts by procedures known to a person skilled in the art.

[0029] N-Formyl-aminometaolts (R' being H) may be prepared from the corresponding aminometaolts by the reaction of the aminometaolts with an alkyl formate such as methyl formate, ethyl formate or butyl formate, with an aryl formate such as phenyl formate, with a mixed anhydride of formic acid and another carboxylic acid such as acetic formic anhydride or with formic acid.

[0030] N-Acyl-aminometaolts (R' being substituted or unsubstituted C₅₋₇-alkyl or substituted or unsubstituted C₅₋₇-alkenyl) may be prepared by the reaction of the aminometaolts with the appropriate acyl chloride, carboxylic acid alkyl ester, carboxylic acid aryl ester, carboxylic acid anhydride or carboxylic acid. N-Acetyl aminometaolts may be prepared from the corresponding aminometaolt with reaction with acetyl chloride, acetic anhydride or acetic acid, for example.

[0031] N-Alkoxycarbonyl-aminometaolts (R' being OR and R' being unsubstituted C₅₋₇-alkyl) may be prepared by reacting the corresponding aminometaolt with the appropriate alkyl chloroformate, alkyl azidocarbonate or with the appropriate dialkyl-dicarbonate. N-Tert-Butyloxycarbonyl-aminometaolts may be prepared by reacting the corresponding aminometaolt with di-tert-butylyl dicarbonate, for example.

[0032] The aminometaolt may be prepared from the corresponding pentoses such as D-arabinose, L-arabinose, D-xylene, L-xylene, D-ribose, L-ribose, D-lyxose and L-lyxose by procedures known to a person skilled in the art. The pentose may be reacted with a primary or secondary amine or with hydroxylamine to the corresponding imine or oxime,
which may be catalytically hydrogenated to afford the aminotetraol. 1-Amino-1-deoxy-D-arabinitol may be pre-
pared from D-arabinose and 1-amino-1-deoxy-L-arabinitol may be prepared from L-arabinitol by reacting the ap-
propriate enantiomer of arabinose with benzylamine under reducing conditions and catalytically hydrogenating
the obtained corresponding N-benzyl-1-amino-1-deoxy-arabinit-
ol.

[0033] The biooxidation step can be performed with any microorganism capable of oxidizing the hydroxyl group at
C-4 of the N-protected aminotetraols to a keto group. The microorganism may be a bacterium or a fungus. Suitable
bacteria are of the genera Gluconobacter, Acetobacter or Corynebacterium. Suitable fungi are of the genera Metschini-
kowia, Candida or Saccharomyces.

[0034] Preferred microorganisms are bacteria of the gen-
era Gluconobacter or Acetobacter.

[0035] More preferred microorganisms are of the species Gluconobacter oxydans. Bacteria of the species Gluco-
 nobacter oxydans shall also refer to the subspecies Gluconobacter oxydans ssp. suboxydans (formerly known as Acketo-
bacter suboxydans or Acetomonas suboxydans) and Gluconobacter oxydans ssp. oxydans (formerly known as Acetomonas oxydans).

[0036] Most preferred microorganisms are selected from the
group of strains consisting of Gluconobacter oxydans ssp. suboxydans with the designations DSM 2003 (DSM
14076), DSM 2343 and DSM 50049 (ATCC 621).

[0037] These strains can be obtained from the Deutsche
Sammlung für Zellkulturen und Mikroorganismen GmbH
(DSMZ), Mascheroder Weg 1b, D-38124 Braunschweig, Ger-
many. The strain Gluconobacter oxydans ssp. suboxy-
dans DSM 2003 was deposited on 23.02.2001 at the DSMZ
under the terms of the Budapest Treaty with the designa-
tion DSM 14076.

suboxydans with the designations DSM 2003 (DSM 14076),
DSM 2343 and DSM 50049 (ATCC 621) shall also refer to
mutants thereof belonging to the species Gluconobacter oxydans ssp. suboxydans and being able to oxidize the
hydroxyl group at C-4 of the N-protected aminotetraol to a
keto group. Such mutants may be obtained by induced or
spontaneous mutation of the above bacterial strains, fol-
lowed by the isolation of the obtained mutants and screening
of the isolated mutants for their ability to oxidize the
hydroxyl group at C-4 of the N-protected aminotetraol to a
keto group. Examples for mutations are substitution, inser-
tion or deletion of single or multiple bases or inversion of
DNA segments of the genome. Spontaneous mutations are
naturally occurring mutations due to errors in DNA repli-
cation. Induced mutants may be obtained by procedures
known to a person skilled in the art such as by radiation
(ultraviolet radiation, X-rays or gamma rays), mutagenic
chemicals (ethyl methanesulfonate, nitrite or bromouracil)
or by polymerase chain reaction (PCR).

[0039] The microorganisms may be grown on suitable
media comprising nutrients, which serve as carbon, nitrogen
and energy sources, such as soya peptone and yeast extract,
and an inducer of the enzymatic activity catalyzing the
oxidation of the hydroxyl group at C-4 of the N-protected
aminotetraols to a keto group. Suitable inducers of the
enzymatic activity are sugar alcohols such as D-mannitol,
L-mannitol, D-sorbitol, L-sorbitol, galactitol, erythritol,
D-threitol and L-threitol. Preferred inducers are D-sorbitol
and L-sorbitol. The more preferred inducer is D-sorbitol.

[0040] The growth of the microorganisms may be per-
formed at pH 5.0-8.0, preferably at pH 5.5-7.0, and at 20-35°C,
preferably at 28-32°C.

[0041] Preferably, the growth is performed under aerobic
conditions. Precultures may be grown in flasks having a gas
permeable lid and fermentations may be performed in fer-
menters or stirred vessels under gassing with air or oxygen,
for example. The fermentation may be performed at ambient
pressure (ca. 1 bar) or at overpressure up to 3 bar. More
preferably, the growth is performed while keeping the
amount of oxygen in the fermentation broth above 60%
(PPO2/PO2 sat. at atmospheric pressure), preferably above
70%, by gassing with air.

[0042] After growth the microorganisms can be harvested
by e.g. filtration or centrifugation.

[0043] The biooxidation of the N-protected aminotetraol
to the N-protected 1-amino-1-deoxy-pentulose is carried out
with the microorganisms or with cell extracts thereof. The
microorganisms may be employed as resting cells, as immo-
ibilized resting cells or as growing cells. Cell extracts thereof
refer to the crude cell extracts which are obtained by
microbial cell disruption methods known to a person skilled
in the art or by autolysis. Examples of microbial cell
disruption procedures are ultrasound or French press. Pref-
erably, the biooxidation is carried out with a microorganism,
more preferably with resting cells of a microorganism.

[0044] Preferably, the concentration of the N-protected
aminotetraol is 50-250 g/L, more preferably 100-250 g/L,
most preferably 150-250 g/L.

[0045] Preferably, the biooxidation is performed at pH
4.3-6.0, more preferably at pH 4.5-5.5, and at 10-50°C,
more preferably at 10-20°C.

[0046] The biooxidation may be carried out as a batch,
fed-batch or as a continuous process.

[0047] The biooxidation is usually performed for 12-84 h,
preferably for 12-36 h.

[0048] After biooxidation, the cells are removed by e.g.
filtration or centrifugation. The obtained solution may be
used directly in the next step or the N-protected 5-amino-5-
deoxy-D-xylulose may be isolated from the solution
before used in the next step.

[0049] The removal of the N-protective group of N-pro-
tected 5-amino-5-deoxy-pentulose is performed by hydroly-
sis under neutral, alkaline or acidic conditions or by using an
enzyme such as an acylase.

[0050] Preferably, the N-protective group is removed
under alkaline conditions. Examples of suitable bases are
alkali metal hydroxides, alkaline earth metal hydroxides,
ammonium hydroxide, dialkylammonium hydroxides, tri-
alkylammonium hydroxides, tetraalkyl-ammonium hydrox-
ides, alkali metal carbonates or alkali earth metal carbonates.
Examples of alkali metal hydroxides are sodium hydroxide,
potassium hydroxide and lithium hydroxide. Examples of
alkaline earth metal hydroxides are calcium hydroxide and
barium hydroxide. An example of dialkylammonium
hydroxides is diisopropylammonium hydroxide. An example of trialkylammonium hydroxides is triethylammonium hydroxide. An example of tetraalkylammonium hydroxides is tetrabutylammonium hydroxide. Examples of alkaline earth metal carbonates are calcium carbonate and barium carbonate.

More preferably the N-protective group is removed with 1-2 mol equivalents of an alkali hydroxide in respect to N-protected 5-amino-5-deoxy-pentulose.

Most preferably the N-protective group is removed with 1-2 mol equivalents of sodium hydroxide or potassium hydroxide in respect to N-protected 5-amino-5-deoxy-pentulose.

Suitable hydrogenation catalysts include nickel or noble metals such as palladium, rhodium or platinum as the reducing agent. Examples of hydrogenation catalysts are Raney nickel, palladium on charcoal, palladium on barium sulfate, palladium on calcium carbonate, platinum on charcoal, rhodium on charcoal and rhodium on aluminium oxide. The noble metal content of the catalyst is 1-20% (w/w), preferably 4-10% (w/w). Preferably, the hydrogenation catalyst is a palladium catalyst, more preferably the hydrogenation catalyst is palladium on charcoal.

The amount of catalyst used in the hydrogenation may be 0.1-100% (weight of catalyst/weight of 5-amino-5-deoxy-pentulose), preferably 0.5-20%, more preferably 0.5-10%.

The solvent used in the hydrogenation may be water or a water-miscible alcohol such as methanol, ethanol, propanol or isopropanol or mixtures of water and a water-miscible alcohol, preferably water or mixtures of water and a water-miscible alcohol, preferably water.

The hydrogenation may be carried out at 0-120°C, preferably, at 15-40°C, more preferably at 20-30°C, and at a hydrogen pressure of 0.5-100 bar, preferably of 2-10 bar, more preferably at 4-6 bar.

The reaction time for hydrogenation is usually 1-50 h, preferably 5-40 h, more preferably 10-30 h.

The hydrogenation usually provides either the (2R)— or the (2S)-2-hydroxymethyl-pyrrrolidine-3,4-diol in high diastereomeric excess (d.e.), preferably >80% d.e., more preferably >90% d.e.

After the hydrogenation, the catalyst can be removed by e.g. filtration. The product 2-hydroxymethyl-pyrrrolidine may be purified by ion exchange chromatography.

The removal of the N-protective group and the catalytic hydrogenation can be separate process steps or can be performed in the same process step. Preferably the removal of the N-protective group and the catalytic hydrogenation are performed in the same process step.

Also part of the present invention is a process for the preparation of 2-hydroxymethyl-3,4-diol comprising the steps of

a) removing the N-protective group of N-protected 5-amino-5-deoxy-pentulose of the formula

\[
\text{OH} \\
\text{R} \\
\text{N} \\
\text{H} \\
\text{O} \\
\text{H} \\
\text{OH} \\
\text{OH}
\]

b) catalytically hydrogenating said 5-amino-5-deoxy-pentulose (III) to produce the corresponding (2R)— and/or (2S)-hydroxymethyl-pyrrrolidine-3,4-diol of the formula

\[
\text{HO} \\
\text{N} \\
\text{H} \\
\text{O} \\
\text{H} \\
\text{H} \\
\text{OH} \\
\text{OH}
\]

and


All definitions given for the preparation of 2-hydroxymethyl-pyrrrolidine-3,4-diol from N-protected aminoalcohol (I) apply accordingly, when appropriate, to this process.

Another object of the present invention is a process comprising the step of oxidizing an N-formyl-aminotetraol of the formula

\[
\text{HO} \\
\text{N} \\
\text{H} \\
\text{OH} \\
\text{OH}
\]
or a salt thereof with a microorganism or a cell-free extract thereof to produce the corresponding N-formyl-5-amino-5-deoxy-pentulose of the formula

![Chemical Structure]


[0071] Preferred N-formyl-amino-diolts are selected from the group consisting of N-formyl-1-amino-1-deoxy-D-arabinotols and N-formyl-1-amino-1-deoxy-L-arabinotols.

[0072] The more preferred N-protected amino-diolts are N-formyl-1-amino-1-deoxy-D-arabinotols.

[0073] All definitions given for the preparation of 2-hydroxyethyl-2-pyrrolidin-3,4-diol from N-protected amino-diolts (I) apply accordingly, when appropriate, to this process.

[0074] The compounds N-formyl-1-deoxy-1-amino-D-arabinitol and N-formyl-1-deoxy-1-amino-L-arabinitol are also part of the invention.

**EXAMPLE 1**

Preparation of N-formyl-1-amino-1-deoxy-D-arabinitol

[0075] 1-Amino-1-deoxy-D-arabinitol (50.0 g, 330 mmol) was dissolved in methanol (1300 mL) by warming up to 35°C. After cooling to 20°C, methyl formate (43.6 g, 725 mmol) was added and the solution was stirred at 20°C in a double-walled flask with mechanical stirrer and heating-cooling system. After 2 h a white solid precipitated. After a reaction time of overall 3 h, the mixture was cooled to 0°C in 1 h under stirring. For work up, the obtained white suspension was filtered off and washed twice with cold methanol (35 mL each). Drying of the precipitate at 40°C and 25 mbar afforded N-formyl-1-amino-1-deoxy-D-arabinitol (82%).

**EXAMPLE 2**

Preparation of N-formyl-1-amino-1-deoxy-L-arabinitol

[0076] The formylation was carried out as described in example 1, except that 1-amino-1-deoxy-L-arabinitol was used as starting material and N-formyl-1-amino-1-deoxy-L-arabinitol was obtained.

**EXAMPLE 3**

Biomass Preparation

[0077] Medium A was prepared by dissolving yeast extract (3 g/L), D-sorbitol (60 g/L) and soya peptone (5 g/L) in deionized water and adjusting the pH to 6.3 ± 0.3 with NaOH. Medium A was placed in 3 Erlenmeyer flasks (100 mL each), sterilized (121°C, 20 min, 1 bar) and inoculated with *Glucobacter oxydans* ssp. suboxydans DSM 2003 (DSM 14076) (1 mL each). The precultures were incubated at 30°C and 200 rpm for 24 h. Medium A (11.5 L) was placed in a 30 L fermentor, sterilized (121°C, 30 min, 1 bar) and inoculated with preculture (200 mL). The fermentation was performed at 28°C, at pH 6.3 keeping the amount of oxygen in the fermentation broth above 70% P<sub>2</sub>O<sub>2</sub> sat at atmospheric pressure) by gassing with air. After ca. 13 h the fermentation broth was cooled and filtrated at 4°C. The cells were washed by ultrafiltration with aqueous MgSO<sub>4</sub> (20 mM) and concentrated. The concentrated cell suspension was diluted with aqueous MgSO<sub>4</sub> (20 mM) to OD<sub>650nm</sub> 200 and stored at 4°C.

**EXAMPLE 4**

Biooxidation of N-formyl-1-amino-1-deoxy-D-arabinitol

[0078] N-Formyl-1-deoxy-1-amino-D-arabinitol (20 g, 111 mmol) was dissolved in deionized water (100 mL). Concentrated cell suspension of *Glucobacter oxydans* ssp. suboxydans DSM 2003 (DSM 14076) prepared as described in example 3 was added and the pH was adjusted to 5.0. The biooxidation was performed at 15°C and pH 5 under shaking. After 24 h the starting material was almost quantitatively converted to N-formyl-5-amino-5-deoxy-D-xylulose as determined by TLC. The cells were removed by ultrafiltration and the obtained solution was stored until hydrogenation.

**EXAMPLE 5**

Biooxidation of N-formyl-1-amino-1-deoxy-L-arabinitol

[0079] The biooxidation was carried out as described in example 4, except that N-formyl-1-amino-1-deoxy-L-arabinitol was used as starting material. N-Formyl-1-amino-1-deoxy-L-arabinitol was converted to N-formyl-5-amino-5-deoxy-L-xylulose.

**EXAMPLE 6**

Catalytic hydrogenation of N-formyl-5-amino-5-deoxy-D-xylulose

[0080] An aqueous solution of N-formyl-5-amino-5-deoxy-D-xylulose (100 g, 111 mmol) obtained as described in example 4, KOH (1 M, 111 mL, 111 mmol) and Pd/C (4% with 53.4% water (Degusa-Hüls), 1.0 g) were mixed in deionized water (50 mL) in a Hastellov® 1 L autoclave with heating-cooling system. The reaction mixture was purged three times with nitrogen (5 bar) and then three times with H<sub>2</sub> (5 bar). The reaction vessel was pressurized with H<sub>2</sub> (5 bar). After 20 h stirring at 23°C, the reaction mixture was purged three times with N<sub>2</sub>. After filtration over Celite®B, a brownish solution (183 g) was obtained. The solution was purified with an ion exchange resin column (diameter 3 cm, height 20 cm, Dowex® 50 Wx8 H<sub>4</sub>, 100-200 mesh). The resin was activated with aqueous HCl (7%) and then washed with water to a pH of 3-4. After product loading the column was purged 5 times with water (168 mL each time), then
eluted with aqueous NH₃ (5%, 114 mL) and at the end washed with water (283 mL). The fractions were collected and checked with TLC (solvent system: dichloromethane/water/aqueous ammonia (25%): 17/3/2, detection: KMnO₄/Na₂CO₃:1/1). Rg((2R,3R,4R)-2-hydroxyethyl-pyrrolidine-3,4-diol): 0.2-0.4, Rg(N-formyl-5-amino-5-deoxy-D-xylulose): 0.83). After pooling of identical fractions and removal of water, (2R,3R,4R)-2-hydroxyethyl-pyrrolidine-3,4-diol (72% starting from N-formyl-1-amino-1-deoxy-D-arabinitol) with a diastereomeric excess of 94.4% as determined by high pressure capillary electrophoresis (HPCE) was obtained.

EXAMPLE 7
Catalytic hydrogenation of N-formyl-5-amino-5-deoxy-L-xylulose

[0081] The hydrogenation was carried out as described in example 6, except that N-formyl-5-amino-5-deoxy-L-xylulose was used as starting material. (25S,3S,4S)-2-hydroxyethyl-pyrrolidine-3,4-diol was obtained.

EXAMPLE 8

[0082] Removal of N-Formyl Protective Group Prior to Catalytic Hydrogenation

[0083] An aqueous solution of N-formyl-5-amino-5-deoxy-D-xylulose (100 g, 111 mmol) obtained as described in example 4, KOH (1 M, 111 mL, 111 mmol) and deionized water (50 mL) were stirred in a Hastelloy® 1 L autoclave with heating-cooling system for 1 h at ca. 23°C. Pd/C (4% with 53.4% water (Degussa-Hüls), 1.0 g) was added and the hydrogenation was performed as described in example 6. (2R,3R,4R-2-hydroxyethyl-pyrrolidine-3,4-diol) was obtained.

1. A process comprising the steps of
   a) oxidizing an N-protected aminotetraol of the formula

   ![Formula I](image)

   or a salt thereof wherein R₁ is H, substituted or unsubstituted C₇-alkyl, substituted or unsubstituted C₆-alkenyl or OR₂, R₂ being unsubstituted C₇-alkyl, with a microorganism or a cell-free extract thereof to yield the corresponding N-protected 5-amino-5-deoxy-pentulose of the formula

   ![Formula II](image)

   R¹ being defined as above,

   b) removing the N-protective group of said N-protected 5-amino-5-deoxy-pentulose (II) to yield the corresponding 5-amino-5-deoxy-pentulose of the formula

   ![Formula III](image)

   and

   c) catalytically hydrogenating said 5-amino-5-deoxy-pentulose (III) to produce the corresponding (2R)- and/or (2S)-hydroxymethyl-pyrrolidine-3,4-diol of the formula

   ![Formula IV](image)

   and/or

   ![Formula V](image)

2. Process according to claim 1 wherein R₁ is substituted or unsubstituted C₇-alkyl or H, preferably H.

3. Process according to one of the preceding claims wherein the N-protective aminotetraol is an N-protected 1-amino-1-deoxy-D-arabinitol or an N-protected 1-amino-1-deoxy-D-arabinitol, preferably an N-substituted 1-amino-1-deoxy-D-arabinitol.

4. Process according to one of the preceding claims wherein the microorganism belongs to the genera Gluconobacter or Acetobacter, preferably to the species Gluconobacter oxydans.

5. Process according to claim 4 wherein the microorganism is selected from the group of strains consisting of Gluconobacter oxydans ssp. suboxydans DSM 2003 (DSM 14076), Gluconobacter oxydans ssp. suboxydans DSM 2349 and Gluconobacter oxydans ssp. suboxydans DSM 50049 (ATCC 621).

6. Process according to one of the preceding claims wherein the oxidation is carried out at a concentration of N-protected aminotetraol of 50-250 g/L, preferably at 100-250 g/L.

7. Process according to one of the preceding claims wherein the oxidation is carried out at pH 4.3-6.0, preferably 4.5-5.5, and 10-50°C, preferably 10-20°C.

8. Process according to one of the preceding claims wherein the N-protective group is removed under alkaline conditions, preferably with 1-2 mol equivalents of an alkaline hydroxide in respect to N-protected 5-amino-5-deoxy-pentulose.

9. Process according to one of the preceding claims wherein the hydrogenation catalyst is a palladium catalyst.
10. Process according to one of the preceding claims wherein the amount of catalyst used in the hydrogenation is 0.5-20% (weight of catalyst/weight of 5-amino-5-deoxy-pentulose), preferably 0.5-10%.

11. Process according to one of the preceding claims wherein the removal of the N-protective group and the catalytic hydrogenation are carried out in the same process step.

12. Process comprising the steps of
   a) removing the N-protective group of N-protected 5-amino-5-deoxy-pentulose of the formula

   \[ \text{R}^1 \end{align*} \]

   to yield the corresponding 5-amino-5-deoxy-pentulose of the formula

   \[ \text{R}^2 \end{align*} \]

   and

   b) catalytically hydrogenating said 5-amino-5-deoxy-pentulose (III) to produce the corresponding (2R)- and/or (2S)-2-hydroxymethyl-pyrrolidine-3,4-diol of the formula

   \[ \text{R}^3 \end{align*} \]

13. Process according to claim 12 wherein the N-protected 5-amino-5-deoxy-pentulose is N-protected 5-amino-5-deoxy-D-xylulose or N-protected 5-amino-5-deoxy-L-xylulose, preferably N-protected 5-amino-5-deoxy-D-xylulose.

14. Process according to claim 12 or claim 13 wherein the N-protective group is removed under alkaline conditions, preferably with 1-2 molar equivalents of an alkaline hydroxide in respect to 5-amino-5-deoxy-pentulose.

15. Process according to one of claims 12 to 14 wherein the hydrogenation catalyst is a palladium catalyst.

16. Process according to one of claims 12 to 15 wherein the amount of catalyst used in the hydrogenation is 0.5-20% (weight of catalyst/weight of 5-amino-5-deoxy-pentulose), preferably 0.5-10%.

17. Process according to one of claims 12 to 16 wherein the removal of the N-protective group and the catalytic hydrogenation are carried out in the same process step.

18. A process comprising the step of oxidizing an N-formyl-aminotetral of the formula

\[ \text{H} \end{align*} \]

or a salt thereof with a microorganism or a cell-free extract thereof to produce the corresponding N-formyl-5-amino-5-deoxy-pentulose of the formula

\[ \text{H} \end{align*} \]

19. Process according to claim 18 wherein the N-formyl-aminotetral is N-formyl-1-amino-1-deoxy-D-arabinitol or N-formyl-1-amino-1-deoxy-L-arabinitol, preferably N-formyl-1-amino-1-deoxy-D-arabinitol.

20. Process according to claim 18 or claim 19 wherein the microorganism belongs to the genera Gluconobacter or Acetobacter, preferably to the species Gluconobacter oxydans.

21. Process according to claim 20 wherein the microorganism is selected from the group of strains consisting of Gluconobacter oxydans ssp. suboxydans DSM 2003 (DSM 14076), Gluconobacter oxydans ssp. suboxydans DSM 2349 or Gluconobacter oxydans ssp. suboxydans DSM 50049 (ATCC 621).

22. Process according to one of claims 18 to 21 wherein the oxidation is carried out at a concentration of N-formyl-aminotetral of 50-250 g/L, preferably of 100-250 g/L.

23. Process according to one of claims 18 to 22 wherein the oxidation is carried out at pH 4.3-6.0, preferably 4.5-5.5, and 10-50° C., preferably 10-20° C.

24. N-Formyl-1-amino-1-deoxy-L-arabinitol of the formula

\[ \text{H} \end{align*} \]

25. N-Formyl-1-amino-1-deoxy-D-arabinitol of the formula